

THE PRIMARY STRUCTURE OF BOVINE GROWTH HORMONE

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1. Introduction

Pituitary growth hormone is a protein hormone of monomer molecular weight about 20 000. Structural studies have been carried out on growth hormones from several species, and have demonstrated substantial specific variation. Several groups have studied the amino acid sequence of bovine growth hormone [1–7]; Santomé et al. [3] gave an almost complete primary structure. However, the sequences proposed by different research groups differ substantially. In this paper, our work on the complete sequence of this hormone is summarized. Particular attention is paid to points of difference from the results of other workers.

The determination of amino acid sequences for growth hormones from different species is of particular interest in relation to the molecular evolution of the hormone [5]. The availability of the sequences of growth hormones from several other species has been of help in confirming that of bovine growth hormone, by homology, but the data acquired for the bovine hormone is, by itself, sufficient to establish the complete primary structure.

2. Experimental

2.1. Materials

Bovine growth hormone was prepared by the method of Wallis and Dixon [8]. Trypsin (TPCK-treated), α -chymotrypsin (3 \times crystallized), pepsin and carboxypeptidases A and B (diisopropyl phosphorofluoridate treated) were from Worthington Biochemical Corporation. Thermolysin was from Calbiochem and subtilisin from Sigma Ltd.

2.2. Enzymic and cyanogen bromide digests; preparation of peptides

Before enzymic digestion, growth hormone was modified by (a) performic oxidation [9], (b) reduction followed by aminoethylation [10] or (c) carboxymethylation of methionine residues [6], sometimes followed by performic oxidation [9].

Various digests of protein so modified were prepared, using trypsin, chymotrypsin, thermolysin, pepsin or trypsin plus chymotrypsin. Details of the preparation of some of these digests have been presented elsewhere [6, 11, 12]. In each case, digestion was performed in a volatile buffer, for 6–8 hr at 37°C, using an enzyme:protein ratio of 1:50. After digestion, insoluble peptides were removed, and the soluble fraction was freeze-dried.

The soluble peptides were fractionated by one of two main methods. The first involved gel filtration on Sephadex G-50 and G-25, followed by further purification of peptides by high voltage paper electrophoresis and paper chromatography. The second purification method involved peptide mapping [6], location of peptides by staining with very weak (0.005%) ninhydrin solution and preparation of peptides directly from these maps by elution with 6 N HCl. This second method allowed small quantities (up to 100 nmoles) of peptide to be prepared quite easily.

Most of the enzymic digests gave an insoluble fraction. The insoluble peptides after trypsin digestion were fractionated by paper electrophoresis in 60% formic acid (in which they were completely soluble) or by paper chromatography in n-butanol/acetic acid/water (3/1/1, by volume), in which some of the peptides were soluble.

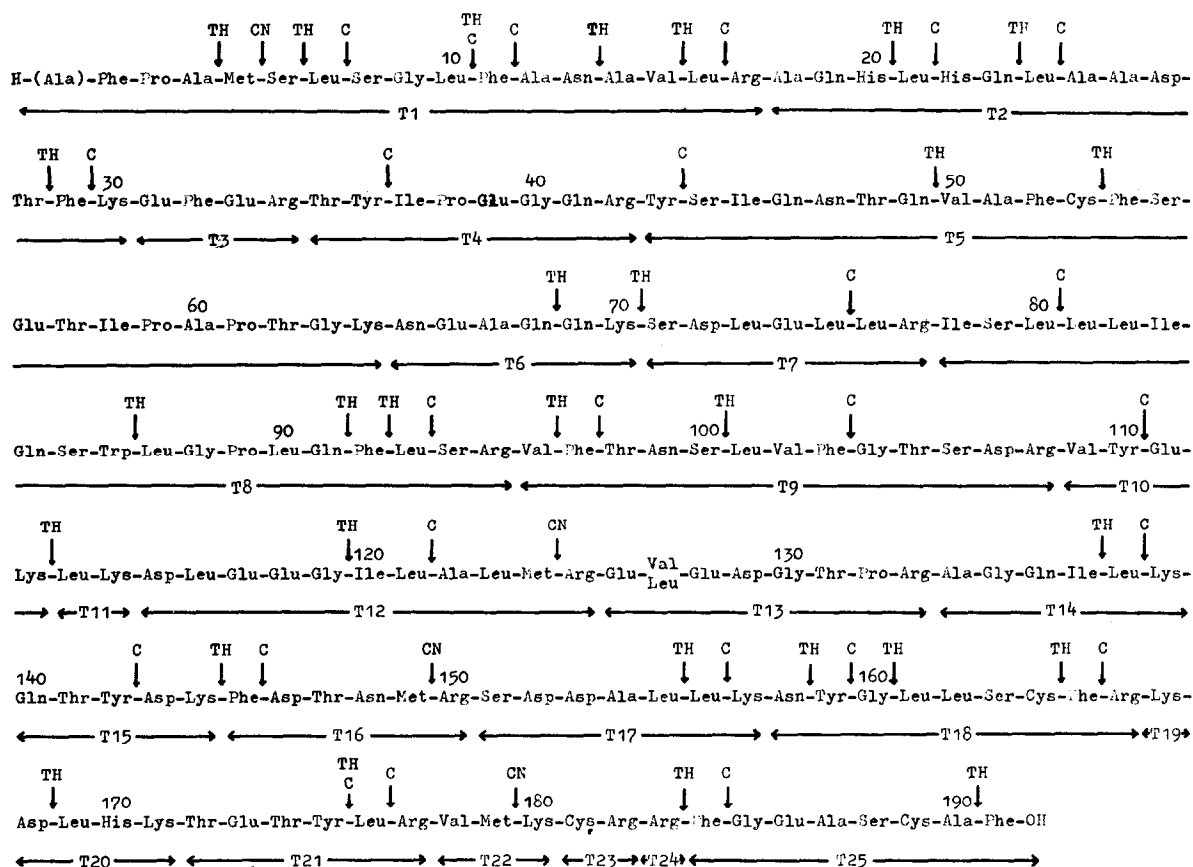


Fig. 1. The primary structure of bovine growth hormone. T1, T2, etc. indicate peptides derived from tryptic digests of the protein. C, TH, CN indicate points of cleavage by chymotrypsin, thermolysin and cyanogen bromide, respectively (as indicated by peptides isolated from digests of the whole protein). Disulphide bridges connect residues Cys 53-Cys 164 and Cys 181-Cys 189. Heterogeneity at residue 1 and residue 127 is discussed in the text.

Growth hormone was also fragmented with cyanogen bromide, using conditions described previously [4]. The peptides produced were fractionated by gel filtration.

2.3. Characterization of peptides

Amino acid compositions were determined, after hydrolysis in 6 N HCl (110°C, 24 hr, sealed tube; a trace of phenol was added to protect tyrosine), on a Locarte amino acid analyser.

N-terminal amino acid residues were determined by the 'dansyl' method [13] and N-terminal sequences by the 'dansyl'-Edman technique [14]. C-terminal residues and sequences were investigated in some cases by the use of carboxypeptidases A and/or B.

For characterization of large peptides, further enzymic digestion was used in many cases, using subtilisin or one of the enzymes already mentioned. Such secondary digests were fractionated by the methods outlined. In some cases unfractionated digests were investigated, using the 'dansyl'-Edman method, and the approach advocated by Gray [15].

Location of amide groups was carried out mainly by investigating the mobility of peptides on electrophoresis at pH 6.5. In some cases the mobility of 'dansyl'-peptides during 'dansyl'-Edman degradation was investigated; in other cases amide assignments were based on the products of hydrolysis of peptides by leucine aminopeptidase or carboxypeptidase.

3. Results

The sequence derived for bovine growth hormone is shown in fig. 1. The polypeptide chain contains 191 amino acid residues, though one of these, the N-terminal alanine, is missing from half of the polypeptide chains [4].

The bulk of the detailed sequence work was carried out on peptides derived from tryptic digests of the hormone. Most of the tryptic peptides were soluble, and could be fractionated quite readily. However, 3 tryptic peptides, T1, T5 and T8, were insoluble and proved difficult to fractionate, though T1 could be solubilized when prepared from growth hormone in which methionine residues had been carboxymethylated, while T5 was split and solubilized when prepared from reduced and aminoethylated hormone. T1, T5 and T8 could be fractionated by paper electrophoresis in 60% formic acid, but yields were poor.

The order of the tryptic peptides in the growth hormone molecule was determined by analysis of peptides from chymotrypsin, thermolysin and cyanogen bromide digests. The results from the last of these agreed well with those of Fellows and Rogol [1]. The investigation of peptides from these digests provided further information about regions of the sequence which had not been fully clarified from the tryptic peptides. The products of a trypsin plus chymotrypsin digest [6] also helped in analysis of these difficult regions of the sequence. The order of tryptic peptides derived from 'overlapping' peptides agreed completely with that given by homology with human or sheep growth hormones.

Heterogeneity of the growth hormone sequence was detected at two positions: the N-terminus (half the polypeptide chains lack the N-terminal alanine residue [4]) and residue 127 (where, in agreement with earlier reports [1, 3, 16], both leucine and valine were found, in a ratio of about 2:1). No evidence was found for heterogeneity elsewhere in the molecule, but it certainly cannot be completely ruled out on the basis of the present data.

There are two cystine residues in bovine growth hormone, and therefore, two disulphide bridges. The location has been shown [1, 3] to be Cys 53—Cys 164 and Cys 181—Cys 189, and this was confirmed in the present work.

4. Discussion

Several groups have reported studies on the amino acid sequence of bovine growth hormone. The most nearly complete sequence published previously is that of Santomé et al. [3]. There are several differences between their sequence and that presented here. The main features concern the sequences of tryptic peptides T2 (differing at about 7 positions), T4 (differing at 2 positions), T8 (about 7 differences) and T11—T12 (residues 113—124). In addition amide residues have been located at positions 48 and 69 whereas Santomé et al. placed free carboxyl groups at these positions.

Fellows et al. [2] determined the N-terminal sequence of bovine growth hormone, as far as residue 44, using an amino acid sequenator. Their results are in complete agreement with those presented here. They also reported the sequence of residues 124—191, which is also in full agreement with that determined in the present work. The sequence given by Dayhoff [7] is based on the results of all three groups working on this hormone.

Li et al. [17] have presented a sequence for ovine growth hormone, which is very similar to that reported here for the bovine hormone. The only differences are at positions 99 (aspartic acid in ovine growth hormone replaces asparagine in the bovine hormone) and 130 (valine in ovine growth hormone, glycine in the bovine hormone). In addition, the valine/leucine microheterogeneity at residue 127 may not be present in the ovine hormone. An extended sequence for the C-terminal region of ovine growth hormone has also been presented by Bellair [18]; this agrees completely with that of Li et al. [17]. Fernández et al. [19] have presented a partial sequence for ovine growth hormone, but this differs somewhat from that of Li et al. [17]. Dayhoff [7] summarizes other work on the ovine growth hormone sequence. In addition, Seavey et al. [16] also identified the difference at residue 130, from peptide mapping. Whether the difference at residue 99 is a real one must be in doubt, because ovine and bovine growth hormones behave in a very similar way on isoelectric focussing and polyacrylamide gel electrophoresis [20]; one would expect that a single charge difference due to an aspartic acid/asparagine substitution would have a substantial effect on the isoelectric points of the hormones [12].

The comparison of ovine and bovine growth hormone sequences thus confirms earlier ideas that these two hormones are very similar; they differ only at one or two residues. Bovine growth hormone differs very substantially, however, from the human hormone [21, 22] (at 35–40% of all residues) and quite substantially from porcine growth hormone [23] (at least 10% of all residues). The sheep and cow are of course quite closely related, but the degree of similarity between their growth hormones is surprising in view of the differences seen between the human, pig and bovine hormones. The results confirm the idea, discussed previously [5], that rates of molecular evolution in this group of protein hormones have varied widely.

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